



Variation in ploidy level and genome size of *Cynodon dactylon* (L.) Pers. along a latitudinal gradient

Jingxue Zhang · Miaoli Wang · Zhipeng Guo ·
Yongzhuo Guan · Yuxia Guo · Xuebing Yan

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Abstract Knowledge of ploidy level and genome size in a germplasm collection is critical before studying genetic diversification of different environmental range in grasses and other plants. We assessed the geographic patterns in ploidy level and genome size of 216 individuals of *Cynodon dactylon* (L.) Pers. (common bermudagrass) by flow cytometry of accessions sampled from 16 geographic sites along a latitudinal gradient from 22°35' N to 36°18' N across China. Flow cytometry histograms combined with mitotic chromosome observations results show that tetraploids were the most frequent ploidy level, constituting 44.91% of all individuals. Nuclear DNA contents were 2.384, 2.419, 2.437, 2.873 and 3.288 pg/2C for the diploid, triploid, tetraploid, pentaploid and hexaploid, respectively. Higher proportions of polyploid individuals were observed within populations at the highest and lowest latitudes. In addition, monoploid genome size of *C. dactylon* progressively increased with increasing ploidy level. Temperature and precipitation had the

influence on ploidy level for all the sites. The relationship between ploidy level and geographic distribution for *C. dactylon* will facilitate the utilization of this species for biological and genetic research.

Keywords grass · evolution · latitude · polyploidy · genome variation · *Cynodon dactylon* · common bermudagrass

Introduction

The wind-pollinated *Cynodon dactylon* (L.) Pers. (common bermudagrass) is widely distributed in temperate and tropical regions of the world and comprises diploid ($2n = 2x = 18$) and polyploid cytotypes which are used for turfgrass, pasture, forage, soil stabilization, and remediation in arid and semiarid regions (Taliaferro et al. 2004). Of the 690 *C. dactylon* germplasm accessions collected across Australia, the most commonly observed ploidy levels were tetraploids (61%) followed by triploids (14%), diploids (11%), pentaploids (0.003%) and hexaploids (0.01%; Jewell et al. 2012). These five groups of ploidy levels were also found in Turkey among 182 *C. dactylon* accessions (Gulsen et al. 2009). Monoploid genome size (known as the C-value) is used to refer to the amount of DNA contained in the cell nucleus, which is typically broadly constant within an organism (Greilhuber et al. 2005; Swift 1950). Genome size is an important characteristic for a species that is related in part to the ploidy level, plant physiology, ecology and genome evolution (Heslop-Harrison

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J. Zhang · M. Wang · Z. Guo · Y. Guan · Y. Guo (✉)
College of Animal and Veterinary Science, Henan Agricultural University, Zhengzhou 450002, China
e-mail: yuxiaguo@163.com

X. Yan (✉)
College of Animal Science and Technology, Yangzhou University, Yangzhou 225000, China
e-mail: yxbbjzz@163.com

1995). Genome size studies of polyploids have contributed to improving the knowledge of the process of polyploid formation (Bures et al. 2004; Poggio et al. 2014; Castelli et al. 2017). Many studies of other turfgrasses using laser flow cytometry have demonstrated that nuclear DNA content is closely correlated with chromosome number, and thus ploidy level (Johnson et al. 1998; Eaton et al. 2004). The nuclear DNA content and ploidy levels were observed in 43 native *C. dactylon* accessions collected from the west to east coast of Korea (Kang et al. 2008). Genome sizes and ploidy levels of warm-season grass species, such as *Cynodon* spp., *Paspalum* spp. and *Zoysia* spp., have been described using flow cytometry (Jarret et al. 1995; Johnson et al. 1998; Kang et al. 2008; Schwartz et al. 2010).

Ramsey (2011) concluded that polyploidy can have a role in adaptation to a new environment. Polyploidy appears to be positively associated with latitude, elevation and recent deglaciations (Stebbins 1984; Brochmann et al. 2004). Higher proportions of polyploids are generally found at higher latitudes or elevations than related diploids, particularly in herbaceous perennial grasses (Dodson and Dodson 1976; Ehrendorfer 1980). Polyploids have been compared to diploids in terms of their adaptation to various environments. Different ploidy levels in *Stenotaphrum secundatum* (Walt.) Kuntze. (St. Augustine grass) lead to adaptive polymorphism. Polyploids have increased environmental robustness, and an increased potential for specific adaptation, mating system shifts and increasing self-compatibility of polyploids can facilitate higher rates of establishment in new habitats (Husband et al. 2008). Genome size has been correlated with the environment and the geographical distribution of species (Bennett 1976, Bennett 1987). Numerous studies show that variation in DNA C-value is strongly correlated with many phenotypic features of cells and organisms (Bennett and Leitch 1995). Genome size has the influence on important ecological characteristics of plant species in natural habitats, for example the timing of spring growth, cell size and rate of leaf expansion in early season growth, frost resistance, and xeric conditions (Grime et al. 1985; Poggio et al. 1989; Macgillivray and Grime 1995). Knowledge of polyploidy and genome size in *C. dactylon* in China may partly reflect their increased genetic variability. There are abundant *C. dactylon* distributed in the southern half of China, but little plant selection for use in conservation work of *C. dactylon* has focused on ploidy level and

genome size. So, one specific experiment was conducted and *C. dactylon* individuals were sampled along latitudinal gradients to answer the question. The objectives of this study were to (i) determine the ploidy level and DNA content of 216 *C. dactylon* individuals collected from 16 different latitudes from 22°35' N to 36°18' N across China; and (ii) explore the latitudinal pattern of polyploid and DNA content.

Material and methods

Plant material

A total of 216 *C. dactylon* germplasm accessions were collected from 16 sites along a latitudinal gradient between 22°35'N and 36°18'N, spanning most of the species' North-South distributional range across China (Fig. 1). At each collection site, twenty plants composed of the roots and the stem were sampled at random at least 50 metres apart and later planted at an experimental farm. Total annual precipitation, mean annual temperature, annual maximum and minimum temperature were provided by the China Meteorological Administration for the collection locations of the plants (Table 1).

Flow cytometry

Plant individuals were grown in 1 × 1 m plots and mown once after one month of growth without fertilization, irrigation and pesticides at an experimental farm in Zhoukou, Henan Province. Each plot was separated by a 0.5 m wide gap. Leaves were sampled for the detection of nuclear DNA content. Nuclear DNA content of 216 individuals was determined using a flow cytometer (Cube 8, Partec, Germany) at the Henan Academy of Agricultural Sciences. Nuclei were extracted using CyStain UV precise P (Partec, Munster, Germany). Fifty milligrams of excised leaf material were-ground in 500 µL of extraction buffer (Partec) and the extracted leaf nuclei were stained in 2 mL of propidium iodide (PI) for nuclear DNA content analysis. Following staining, the respective samples were stored at 4°C without light for 30 minutes and then filtered through a 30-µM nylon mesh into a 5-mL test tube. Nuclear DNA content was also

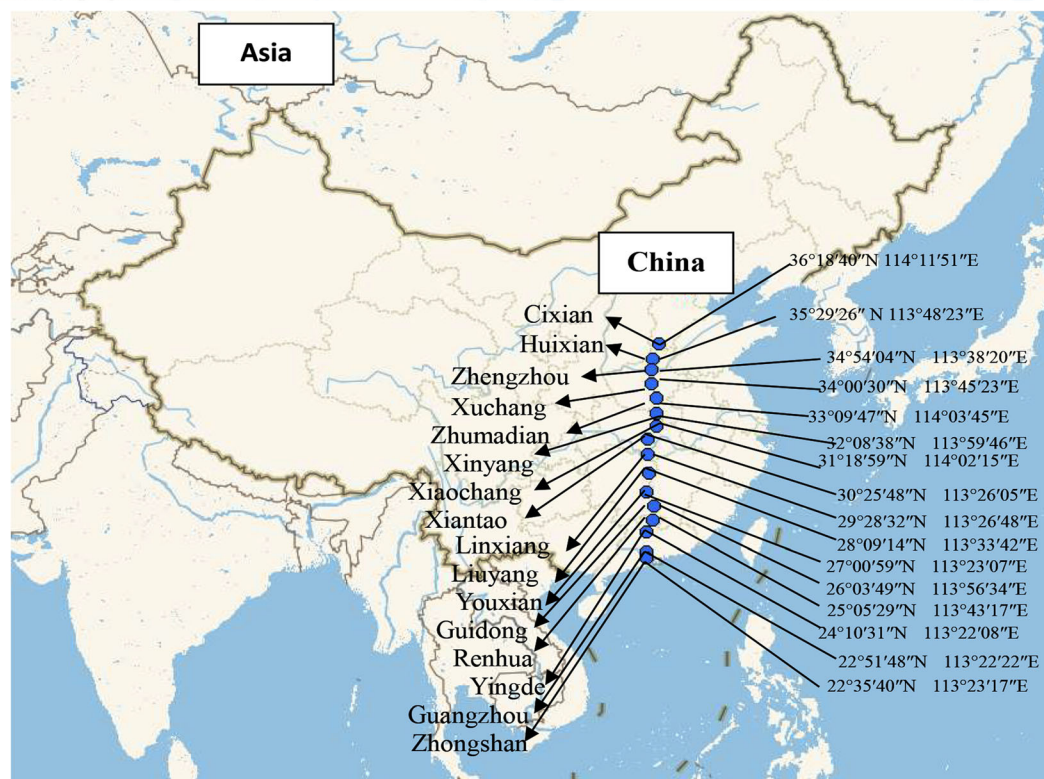


Fig. 1 Distribution of *C. dactylon* accessions collected along the latitude gradient of China. Latitude and longitude of the 16 populations are indicated in the sampling scheme.

determined according to flow cytometry procedures described by Arumuganathan and Earle (1991). The nuclear DNA content of each individual was determined twice using flow cytometry based on at least

1,000 scanned nuclei per sample. CyFlow Cube 13 software was used for the analysis. The following formula was used for converting fluorescence values to DNA content:

$$\text{nuclear DNA content} = \left[\frac{\text{(mean position of sample peak)}}{\text{(mean position of the peak of standard)}} \right] \times \text{DNA content of the standard}$$

Putative ploidy was inferred by comparing DNA contents with thresholds published in Taliaferro et al. (1997). The half-peak coefficient of variation (CV) of the G0/G1 peak was evaluated for each sample to estimate the integrity of the nuclei and variation in DNA staining. The coefficients of variation (CV) for flow cytometry histograms are presented in Supplementary Table 1 and the use of analyses with CV < 4 increased the reliability of the test results. *Pisum sativum* L. (2C = 9.76 pg – Bennett and Smith 1976) leaves were used as the internal standard in the study.

Chromosome counting

After ploidy level analysis using flow cytometry, the chromosome numbers of six individuals for each ploidy level were counted from root-tip smears to verify the precision of the ploidy level measurements obtained by flow cytometry (Arumuganathan et al. 1999). As described by Hanson and Bashaw with minor modifications (Hanson and Oidemeyer 1951; Bashaw and Forbes 1958), root tip samples measuring approximately 1 cm were excised and pretreated in 0.05% colchicine for 4 h, then rinsed in distilled water and placed in a freshly

Table 1 Climatic conditions of *C. dactylon* populations collected from different latitudes in China

Population code	Localities	Latitude (°)	Longitude (°)	Elevation [m a.s.l.]	Habitat	Annual average temperature [°C]	Annual maximum temperature [°C]	Annual minimum temperature [°C]	Annual average precipitation [mm]
1	Cixian	36°18'40"	114°11'51"	130	Roadside	13.4	19.5	7.9	509.2
2	Huixian	35°29'26"	113°48'23"	120	Roadside	14.6	20.3	9.8	586.9
3	Zhengzhou	34°54'04"	113°38'20"	90	Roadside	14.7	20.3	9.9	640.8
4	Xuchang	34°00'30"	113°45'23"	90	Roadside	14.6	20.3	9.9	733.5
5	Zhumadian	33°09'47"	114°03'45"	50	Arable land	15.2	20.4	10.7	990.4
6	Xinyang	32°08'38"	113°59'46"	100	Roadside	15.5	20.4	11.7	1,106.1
7	Xiaochang	31°18'59"	114°02'15"	50	Roadside	16.8	21.3	13.4	1,138.0
8	Xiantao	30°25'48"	113°26'05"	30	Roadside	17.0	21.2	13.8	1,238.6
9	Linxiang	29°28'32"	113°26'48"	60	Roadside	16.8	21.5	13.4	1,582.5
10	Liyang	28°09'14"	113°33'42"	90	Roadside	17.5	22.5	13.8	1,551.3
11	Youxian	27°00'59"	113°23'07"	90	Roadside	18.1	22.6	14.8	1,518.4
12	Guidong	26°03'49"	113°56'34"	810	Slope rangeland	15.8	21.6	12.1	1,742.4
13	Renhua	25°05'29"	113°43'17"	90	Roadside	19.9	25.1	16.5	1,660.9
14	Yingde	24°10'31"	113°22'08"	50	Roadside	21.2	25.8	18.1	1,835.9
15	Guangzhou	22°51'48"	113°22'22"	10	Roadside	22.8	27.2	19.6	1,906.8
16	Zhongshan	22°35'40"	113°23'17"	0	Roadside	22.0	25.9	19.1	1,846.8

Table 2 Estimated average of genome sizes and ploidy levels of *C. dactylon* individuals in 16 collection sites from different latitudes

Ploidy level	Diploid (2n = 18)	Triploid (2n = 27)	Tetraploid (2n = 36)	Pentaploid (2n = 45)	Hexaploid (2n = 54)	Aneuploid
Cixian	0	1	8	0	0	0
Huixian	3	5	10	0	0	1
Zhengzhou	2	1	5	3	4	1
Xuchang	0	0	1	3	11	1
Zhumadian	0	0	5	6	1	1
Xinyang	1	3	10	1	1	2
Xiaochang	1	4	12	0	0	0
Xiantao	2	1	12	1	0	0
Linxiang	4	7	5	0	0	0
Liuyang	0	2	7	0	1	2
Youxian	0	2	11	0	1	0
Guidong	0	1	2	0	0	0
Renhua	1	2	3	1	0	1
Yingde	0	0	1	3	8	2
Guangzhou	0	0	2	2	5	3
Zhongshan	2	7	3	1	0	0
Total	16 (7.41%)	36 (16.67%)	97 (44.91%)	21 (9.72%)	32 (14.81%)	14 (6.48%)
Genome size [1C value/ pg]	1.192 ± 0.151	1.210 ± 0.159	1.218 ± 0.166	1.437 ± 0.298	1.644 ± 0.184	1.477 ± 0.301
Mean DNA content [2C value/pg]	2.384 ± 0.30	2.419 ± 0.31	2.437 ± 0.33	2.873 ± 0.59	3.288 ± 0.36	2.954 ± 0.60

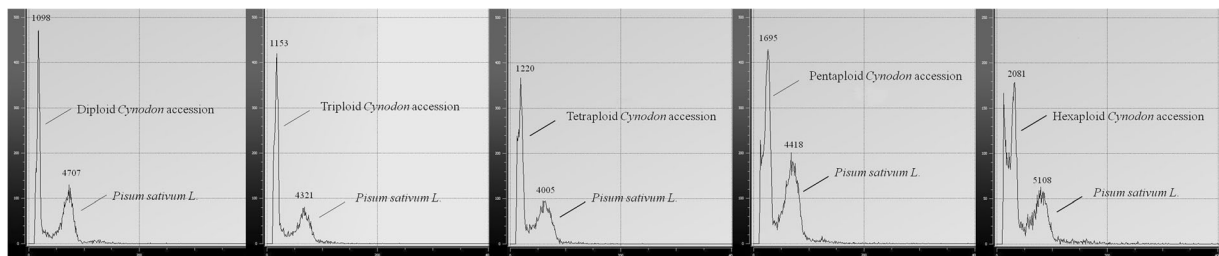
Sample DNA content was calculated by converting fluorescence values according to DNA content of the internal standard. The ploidy levels were inferred by comparing sample DNA content to the previously reported range of respective ploidy levels.

prepared 3:1 ethanol–acetic acid fixation solution for 3 h. Samples were then stored in 70% ethyl alcohol at 4°C for further use. For mitotic analysis, the root tips were hydrolysed in 40% acetic acid for 3 h, then washed in flowing distilled water and stained with acetocarmine for 30 min in the dark. Root tip samples measuring approximately 1 mm were excised, and then stained root tips could be squashed and slides were made. Cells were observed under a Zeiss Scope.A1 fluorescence microscope using a 100× magnification objective and

photographed with an AxioCam MRC5 camera. Images were processed them with ZEN lite 2012 software to determine the number of chromosomes. At least 10 cells at metaphase were observed in each sample.

Data analyses

One-way analysis of variance (ANOVA) was used to test for differences in ploidy level and genome size between the 16 collection sites differing by latitude.

**Fig. 2** Relative positions of the G1 peaks of *C. dactylon* accessions and *Pisum sativum* L.

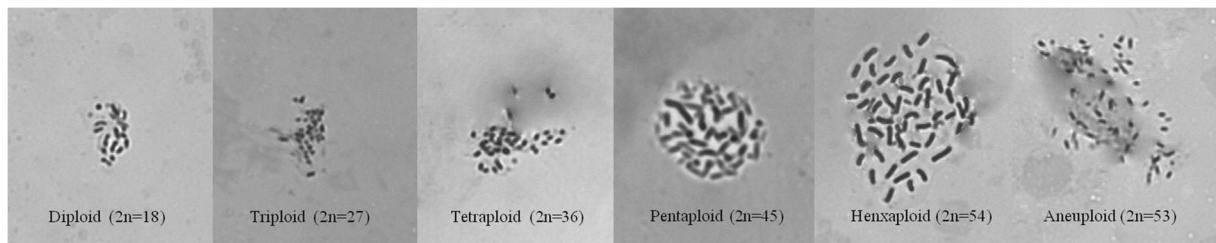


Fig. 3 Pictures of mitotic metaphase chromosomes of *C. dactylon* accessions with different ploidy levels as follows: **a** – diploid ($2n = 18$), **b** – triploid ($2n = 27$), **c** – tetraploid ($2n = 36$), **d** – pentaploid ($2n = 45$), **e** – hexaploid ($2n = 54$), and **f** – aneuploid ($2n = 53$).

The geographical distance matrix was calculated using the arc distance between each pair of sites based on the latitude and longitude of locations. The Mantel correlation value between genome size and geographical distance matrices was calculated by using NTSYSpc version 2.10e. Pearson's correlation coefficient was used to check the relationships between ploidy level and genome size. Regression analysis was used to investigate linear, quadratic and cubic associations between ploidy level, genome size and meteorological characters, considering correlation coefficient (r) values and significance of variables in each model. All statistical analyses were conducted using Statistical Product and Service Solutions (SPSS) 13.0 for Windows (SPSS Inc., Chicago, USA).

Results

Ploidy distribution and genome size

Based on genome size, there were five different ploidy levels: 7.41% of diploids ($2n = 2x = 18$), 16.67% of triploids ($2n = 3x = 27$), 44.91% of tetraploids ($2n = 4x = 36$), 9.72% of pentaploids ($2n = 5x = 45$) and 14.81%

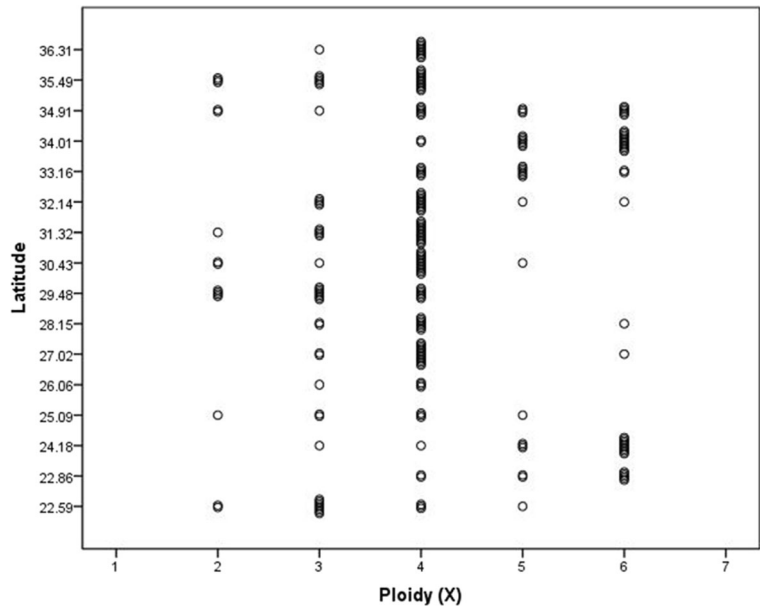
of hexaploids ($2n = 6x = 54$), see Table 2 and Fig. 2. Tetraploid accessions were most prevalent and 6.48% aneuploids were also found. The base chromosome number of *C. dactylon* is nine (Forbes and Burton 1963). The numbers of chromosomes observed under the light microscope agree with ploidy levels previously inferred by flow cytometry (Fig. 3). By contrast, the results pertaining to genome size are incongruent with those of previous studies (Gulsen et al. 2009; Kang et al. 2008; Wu et al. 2006). This divergence may be caused by the different sample sizes examined, differences in the geographic ranges sampled, or different internal standards used in these studies. Ploidy level correlated positively with monoploid genome size ($r = 0.556$; $P < 0.01$). Ploidy levels and genome size values differed significantly along the latitudinal gradient in the ANOVA analysis (Table 3). Ploidy levels in high- and low-latitude populations tended to be greater than those in populations at mid-latitudes in China (Fig. 4). The results of Mantel tests indicated that the nuclear DNA contents distance matrices were not correlated significantly with the geographical distance matrix ($r = -0.01078$, $P = 0.4562$). However, regression analysis indicated that the quadratic association between genome size and latitude was greatly explanatory ($r = 0.341$; $P < 0.01$ – Fig. 5).

Table 3 Ploidy level and genome size of *C. dactylon* for each site along a latitudinal gradient used in analysis of variance (ANOVA)

Traits	Source of variation	Sum of squares	<i>d.f.</i>	<i>F</i>	<i>P</i> -value
Ploidy level	Among sites	127.696	15	12.859	< 0.001
	Within sites	132.411	200		
	Total	260.106	215		
Genome size	Among sites	26.264	15	11.380	< 0.001
	Within sites	29.542	200		
	Total	55.807	215		

Significant at 5% level ($P < 0.05$)

Fig. 4 Distribution of ploidy levels of 216 *C. dactylon* accessions in 16 populations along a latitudinal gradient. Each accession is represented by a little black circle.



Relationship between ploidy level, genome size and climatic conditions

Climatic conditions differ with latitude, annual precipitation and mean temperature at high latitudes being lower than at low latitudes. There were higher ploidy levels at high- and low-temperature sites along the latitudinal gradient. In populations exposed to low precipitation (700–900 mm) and high precipitation (1,800–1,900 mm), there is a high percentage of polyploidy. A quadratic model depicted the

relationship between ploidy levels and temperature ($r = 0.251$; $P < 0.01$), and a cubic association between ploidy level and precipitation was the most explanatory ($r = 0.428$; $P < 0.01$) (Fig. 6). Based on data on genome size and climatic conditions (Fig. 7), regression analysis indicated that quadratic association between genome size and temperature was explanatory ($r = 0.364$; $P < 0.01$) and quadratic association between genome size and precipitation were the most explanatory ($r = 0.375$; $P < 0.01$).

Fig. 5 Regression analysis between genome size of bermudagrass (*C. dactylon*) and latitude

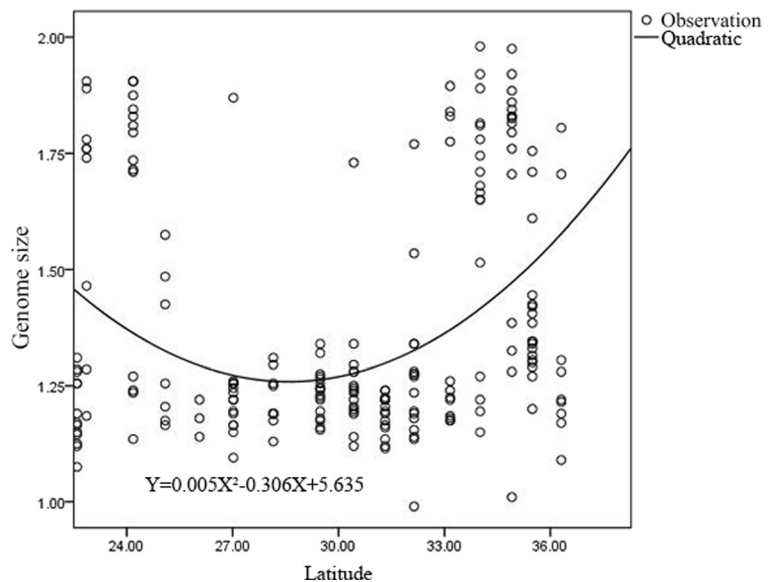
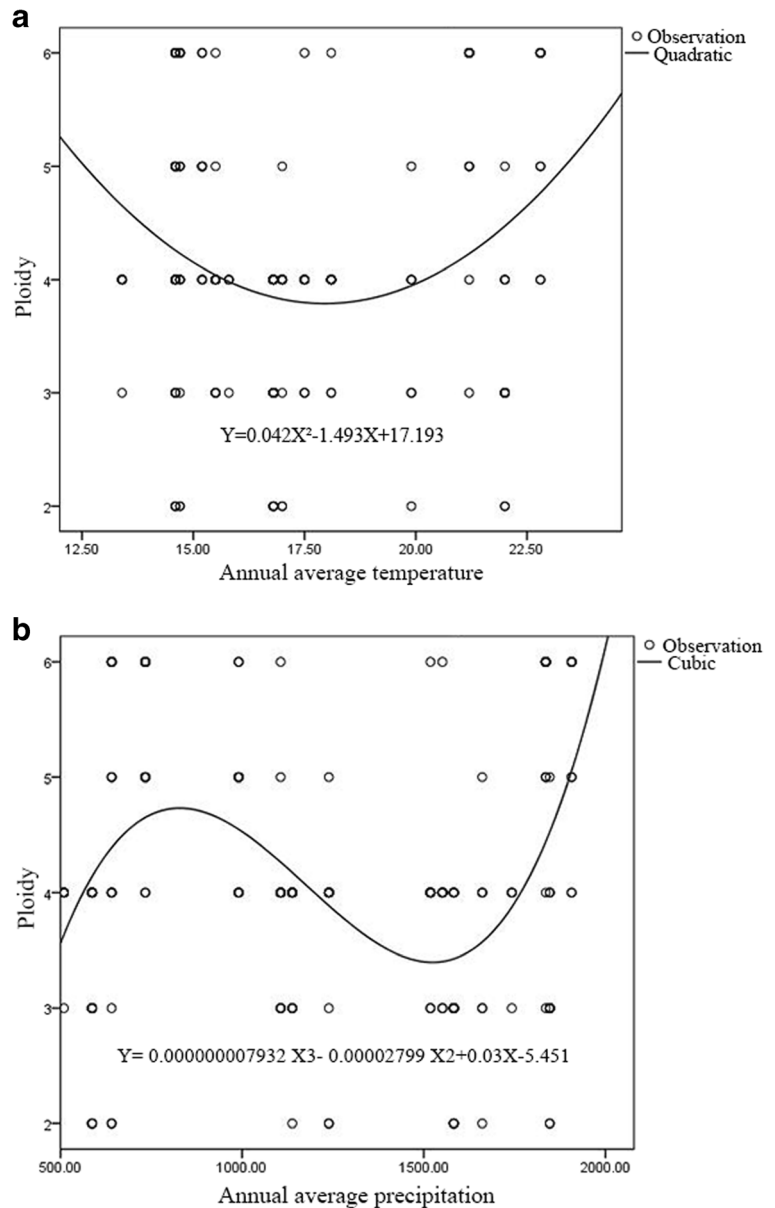


Fig. 6 Regression analysis between ploidy level of bermudagrass (*C. dactylon*) and meteorological characters

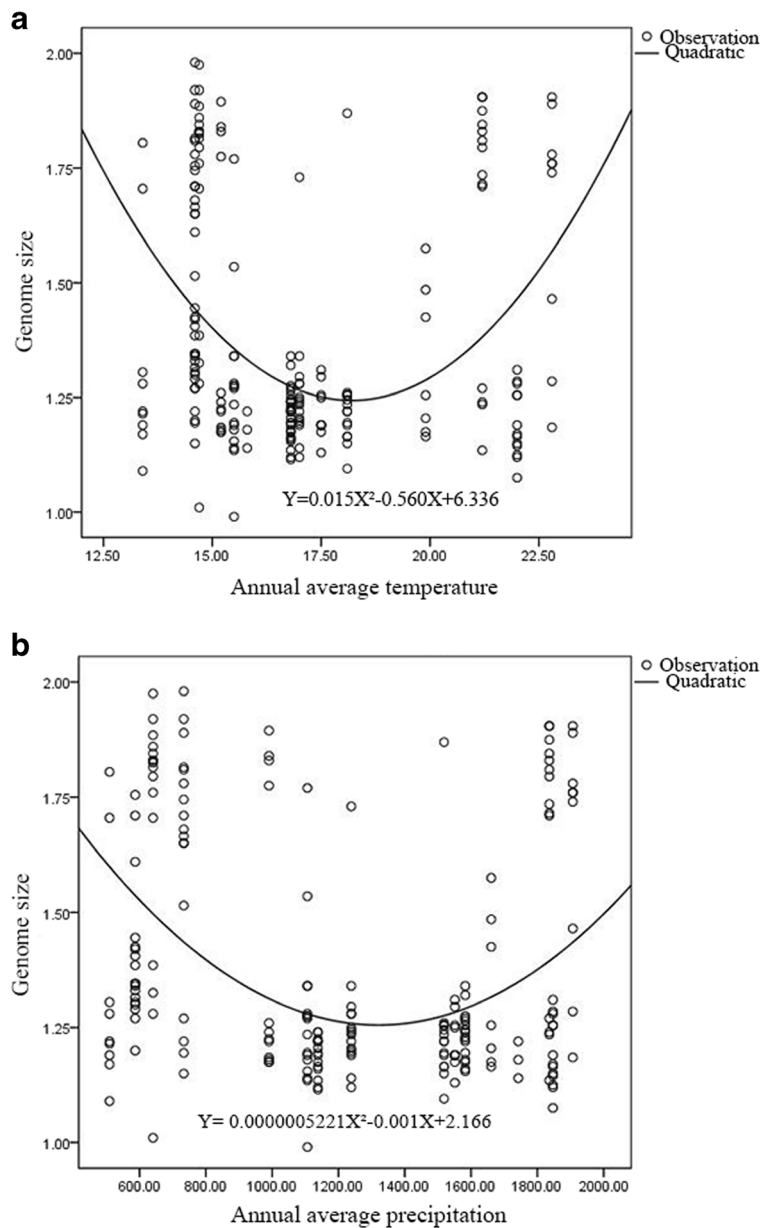


Discussion

As regards the distribution pattern of ploidy levels along latitude gradients, polyploids are better adapted to a broader spectrum of ecological amplitudes (Soltis and Soltis 2000). Polyploids may be linked to increased metabolic and physiological flexibility afforded by gene subfunctionalization, because flexible resource allocation is typical of plants adapted to variable habitats (Aronson et al. 1993). Polyploids have a competitive advantage compared to diploid progenitors because of

their wider ecological amplitude as well as greater potential for higher seed set and faster growth (Maceira et al. 1993; Petit et al. 1999). A geographical correlation of horizontal (latitudinal) distribution with genome size was previously noticed in some plant species, because variation in genome size might have an importance for the adaptation of plants to different environmental conditions (Ohri and Khoshoo 1986; Bottini et al. 2000; Leong-škorničková et al. 2007). The shift from diploids to polyploids of *C. dactylon* along the latitudinal gradient directly corresponds to a notable increase in

Fig. 7 Regression analysis between genome size of bermudagrass (*C. dactylon*) and meteorological characters



monoploid genome size, not as repeatedly observed in several angiosperm groups, where polyploidy often goes along with genome miniaturization (Leitch and Bennett 2004). Among the species of known ploidy level, all 11 weed species whose 4C DNA amounts exceed 40 pg are polyploids, but all seven species with 4C DNA amounts below 1.7 pg are diploids (Bennett 1998). Polyploidization may alter the DNA 1C values of bermudagrass individuals if it is coupled with adaptation to different environments. Varying climatic conditions at different latitudes might favour plants possessing

certain genome sizes and ploidy levels if they are able to tolerate stressful environments. Latitude-related environmental factors, such as temperature and precipitation, acting during growth and developmental stages markedly constrain the distribution of *C. dactylon* with different genome sizes along the latitudinal gradient. Temperature appears to be the most influential factor affecting the distribution of polyploids (Rice et al. 2019). Polyploidy associated with higher latitudes suggests either increased cold tolerance or increased rates of polyploidization in cold climates (Ehrendorfer 1980;

Thompson et al. 2015). Maintaining variation in cold tolerance between populations, this inconsistency in cold-hardiness between diploids and polyploids may be caused by physiological trade-offs between freezing tolerance and growth rate (Bowden 1940; Medeiros et al. 2012). Polyploids with reduced specific leaf area and fewer stomata are more tolerant to drought (Li et al. 1996; Mráz et al. 2014). Tetraploids with wider xylem conduits are likely able to extract more water from drying soil than diploids to avoid a severe reduction in leaf water potential, which facilitates their persistence under drought conditions (Maherali et al. 2009). In addition, some accessions of *C. dactylon* appear to be aneuploids with chromosome numbers of one or two less than the euploid number, which might indicate the presence of aneuploids in *C. dactylon*, as observed in *Paphiopedilum wardii* (Duncan 1945).

Genome size and ploidy level may differentially modify plant traits and interact with environmental factors to influence morphological trait expression (te Beest et al. 2011; Suda et al. 2015). Hexaploid plants of *C. dactylon* such as those of *C. dactylon* cv. ‘Tifton 10’ tend to have thick stolons and coarse-textured long leaves (Wu et al. 2005, 2006). One study has suggested that the fine-textured ecotypes of *C. dactylon* possess lower ploidy levels whereas pentaploids and hexaploids are of the coarse type (Kang et al. 2008). Higher ploidy levels are more adaptable to different conditions because of genetic advantages that facilitate their establishment and persistence (Comai 2005). Polyploidy affects many other genetic and phenotypic characters, and 63% of angiosperms in New Zealand are reported to be polyploids (Hair 1966), which appears to have played an important role in the evolution of the flora. Genome doubling has occurred repeatedly during the evolution of plants, and plants with relatively small genomes have been impacted by polyploidy (Wendel 2000; Seoighe 2003). The discovery that allopolyploids in some plants were formed within the past 200 years could aid the study of the earliest changes in polyploid genome structuring of natural plant populations and the process of plant evolution as a whole (Abbott and Lowe 2004; Soltis et al. 2004). Genome size, which plays an important role in diversification, might also constrain several phenotypic traits and has a significant influence on plant development and ecological performance (Bennett and Leitch 2011; Jersáková et al. 2013; Pellicer et al. 2014). In addition, genome

size variation has been playing an increasingly important role in studies of phylogenetic relationships (Salabert de Campos et al. 2011).

Conclusions

High-ploidy individuals are more common at low and high latitudes and mirror the past distribution history and local adaptations in *C. dactylon*. Monoploid genome size in the species progressively increases with increasing the ploidy level. Accessions of *C. dactylon* with different polyploid levels and genome sizes may further enrich the gene pool and adapt to more different environments that are influenced by latitude-related environmental factors such as temperature and precipitation. Knowledge of the variation in ploidy levels and genome size of *C. dactylon* along the latitudinal gradient may significantly contribute to our understanding of the evolution of this grass.

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Authors' contributions JX analysed and interpreted the data and was a major contributor in writing the manuscript. ML, ZP and YZ helped to collect plants examined in the study. YX and XB contributed to the writing of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Statement of ethics The authors have no ethical conflicts to disclose.

Disclosure statement The authors have no conflicts of interest to declare.

Competing interests The authors declare that they have no competing interests.

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